PREVALENCE OF β_2 GLYCOPROTEIN I DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE MYOCARDIAL INFARCTION

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CERTIFICATE

This is to certify that the dissertation titled **"PREVALENCE OF β_2 GLYCOPROTEIN I DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE MYOCARDIAL INFARCTION"** is the bonafide original work of **DR. M. PAZHANIVEL** in partial fulfillment of the requirements for M.D. Branch – I (General Medicine) Examination of The Tamilnadu DR. M.G.R Medical University to be held in September 2006.The Period of study was from January 2005 to January 2006.

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DECLARATION

I, **DR. M.PAZHANIVEL,** solemnly declare that the dissertation titled **"PREVALENCE OF β_2 GLYCOPROTEIN I DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE MYOCARDIAL INFARCTION"** is a bonafide work done by me at Govt. Stanley Medical College and Hospital during January2005 to January 2006 under the guidance and supervision of my unit chief **Prof. K.RAGHAVAN**, Professor of Medicine.

This dissertation is submitted to The Tamilnadu DR. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (Branch – I) in General Medicine.**

Place: Chennai.

Date:

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CONTENTS

INTRODUCTION

Coronary artery disease is one of the greatest killer diseases of mankind. It is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world.[1] The World Health Organization has drawn attention to the fact that coronary heart disease is our modern epidemic. The prevalence of coronary artery disease has progressively increased in India during the last half century. An aggressive lookout for the evaluation of coronary artery disease is required for some special risk factors apart from the conventional risk factors.

The pathophysiological basis of acute coronary syndromes relies on the existence of vulnerable atherothrombotic plaques within the coronary arteries. Newer inflammatory and immunological mechanisms are emerging for the initiation and progression of atherosclerotic lesions. Anticardiolipin antibodies are a heterogenous family of autoantibodies found to play an important role in the patho physiology of atherosclerosis. They have been implicated as one of the most common acquired protein defects causing thrombosis.[2] These antibodies have been associated with several forms of cardiovascular diseases such as myocardial infarction, stroke, carotid stenosis, and so on. In India, a few studies have been conducted regarding the prevalence and incidence of the anticardiolipin antibodies.

AIM OF THE STUDY

1. To find out the prevalence of beta2-glycoprotein I dependent anticardiolipin antibodies (β_2GPI) of isotype IgG and IgM in patients with ST segment elevation myocardial infarction who were 55 years or younger.

2. To find out the correlation between the conventional cardiovascular risk factors and anticardiolipin antibodies.

REVIEW OF LITERATURE

Acute myocardial infarction is a clinical syndrome that results from an injury to the myocardial tissue, which is caused by an imbalance between the myocardial oxygen supply and demand. Coronary atherosclerosis is an essential part of this syndrome in most of the patients. It is more likely to occur in patients with certain risk factors for this disease. Despite the fact that most cardiovascular events are explained by the conventional risk factors, the search for additional etiologic agents continues. In recent years, a number of new candidate risk factors or markers have been proposed as significant predictors of atherosclerosis and its complications.

MAJOR INDEPENDENT RISK FACTORS [3]

- Advancing age
- Tobacco smoking
- Diabetes mellitus
- Elevated total and low-density lipoprotein
- Hypertension

PREDISPOSING RISK FACTORS

- Abdominal obesity
- Ethnic atherosclerosis
- Family history of premature coronary artery disease

- Obesity
- Physical inactivity
- Psychosocial factors

NOVEL RISK FACTORS FOR ATHEROSCLEROTIC VASCULAR DISEASE [4]

1. Inflammatory markers

- C-reactive protein
- Interleukins (eg, IL-6)
- Serum amyloid A
- Vascular and cell adhesion molecules
- Soluble CD40 ligand
- Leukocyte count

2. Hemostasis / Thrombosis markers

- Fibrinogen
- Von Willebrand factor antigen
- Plasminogen activator inhibitor 1 (PAI-1)
- Tissue-plasminogen activator
- Factors V, VII, and VIII
- D-dimer
- Fibrinopeptide A

- Prothrombin fragment 1+2

3. Platelet-related factors

- Platelet aggregation
- Platelet activity
- Platelet size and volume

4. Lipid-related factors

- Small dense low-density lipoprotein
- Lipoprotein (a)
- Remnant lipoproteins
- Apolipoproteins A1 and B
- High-density lipoprotein subtypes
- Oxidized LDL

5. Other factors

- Homocysteine
- Lipoprotein-associated phospholipase A (2)
- Microalbuminuria
- Insulin resistance
- PAI-1 genotype
- Angiotensin-converting enzyme genotype
- ApoE genotype

- Infectious agents- cytomegalovirus, Chlamydia pneumonia, Helicobacter pylori, Herpes simplex virus

Atherosclerosis is a complex and indolent histopathological process, which is considered to be the most common underlying process in cardiovascular morbidity and mortality. In recent years it has become apparent that in addition to the traditional risk factors for atherosclerosis, this condition is also associated with infectious, inflammatory, and autoimmune factors.[5] Atherosclerosis fulfills all the criteria delineated by Witebsky and Rose to define a condition as autoimmune in nature, and all arms of the immune system (including cellular components, autoantigens, and autoantibodies) play a part in atherosclerosis.[6]

INVOLVEMENT OF CELLULAR COMPONENTS IN ATHEROSCLEROSIS

There is good evidence to suggest that cellular components of the immune system are involved in atherosclerosis. Studies using transgenic murine models show recruitment of mononuclear leucocytes through vascular leucocyte adhesion molecules and chemokines, differentiation of monocytes to macrophages, and endocytosis through scavenger receptors of oxidized low-density lipoprotein in atherogenesis. The importance of T cells in atherosclerosis is emphasized in a study in which CD4+ and CD8+ T cell depletion reduced fatty streak formation in

C57BL/6 mice, indicating that T cells aggravate fatty streak formation.[7] A recent study emphasizes also the importance of specific lymphocytes. Lymphocytes obtained from low-density lipoprotein receptor deficient mice immunized with β_2-glycoprotein I were transferred intraperitoneally into syngenic mice, producing larger fatty streaks in the recipients than in mice receiving lymphocytes from the control mice.[8] T cell depletion of lymphocytes failed to induce this effect. Hence β_2-glycoprotein I reactive T cells could promote atherogenesis. It is also of interest to determine whether atherosclerosis is mainly a T helper 1 or T helper 2 mediated condition.

ASSOCIATION BETWEEN AUTOANTIBODIES AND ATHEROSCLEROSIS

Apart from the involvement of cellular components in atheroscleosis, the evidence also suggests an association between autoantibodies and atherosclerosis.[9] When a pathological state is multifactorial, it is not surprising that many play a part in its pathogenesis.

Anti-oxidized low-density lipoprotein antibodies

Oxidation of low-density lipoprotein probably has an important role in the pathogenesis of atherosclerosis. It is not yet firmly established whether the immune response to oxidized low-density lipoprotein is pro-atherogenic or anti-atherogenic in vivo, or, alternatively, whether it is merely an epiphenomenona for the presence of oxidized low-density lipoprotein. Anti-oxidized low-density

lipoprotein antibodies are raised in patients with early onset peripheral vascular disease, severe carotid atherosclerosis, [10] and angiographically verified coronary artery disease.[11] [12]

In addition, raised levels of oxidized low-density lipoprotein were predictive of carotid atherosclerosis progression, [13] and myocardial infarction occurrence and mortality. [14] It was also found that raised levels of these antibodies occurred in patients with coronary artery disease compared with healthy controls regardless of the amount of coronary calcification. [15]

Anti-heat shock protein (HSP) 60/65 antibodies

Heat shock proteins are a family of proteins that shows a highly homologous sequence between different species from bacteria to man. Sonographic assessment of carotid atherosclerotic lesions showed that subjects with such lesions had significantly raised levels of anti-heat shock protein 65 antibodies compared with controls.[16]

In another study, C57BL/6 mice were injected with either heat shock protein 65 (HSP65), HSP65-rich Mycobacterium tuberculosis, or phosphate buffered saline. Early atherosclerosis was significantly enhanced in mice fed a high cholesterol diet that were immunized with heat shock protein 65. Recently it has been suggested that IL4 has a crucial role in the progression of early atherosclerosis mediated by inflammation, as IL4 knockout mice immunized with

heat shock protein 65 had significantly less fatty streak formation than lesions in C57BL/6 mice.[17]

INCLUSION OF BOTH CELLULAR AND HUMORAL COMPONENTS IN ATHEROSCLEROSIS

Atherosclerosis may also include both cellular and humoral components in its underlying pathophysiology, as occurs in many autoimmune diseases. A recent study determined the role of cellular and humoral immune responses to heat shock protein 65 in murine atherosclerosis. Lymph node cells, splenocytes, and IgG were obtained from the low-density deficient mice immunized with the heat shock protein.[18] Adoptive transfer of heat shock protein reactive lymph node cells increased fatty streak formation in comparison with mice treated with bovine serum albumin primed cells. Similarly, repeated intrapeitoneal administration of IgG from the serum of heat shock protein65 immunised mice enhanced fatty streak formation in mice in comparison with controls.[18] This study provides direct evidence for the pro-atherogenic properties of cellular and humoral immunity to heat shock protein 65, and raises the possibility that both arms of the immune system have a synergistic pro-atherogenic effect.

ANTINUCLEAR ANTIBODIES IN ATHEROSCLEROSIS

Grainger and Bethel provided evidence for the presence of antinuclear antibodies in patients with radiological evidence of advanced atherosclerosis.[19]

Their discussion includes the possibility that these antibodies are merely an epiphenomenon or, alternatively, that they have a pathogenic role in atherosclerosis. Even though this study naturally does not provide answers to that crucial question, their finding itself is important and raises several thoughts and assumptions.

The accelerated atherosclerotic state found in patients with systemic lupus erythematosus and antiphospholipd syndrome might result from the higher frequency of traditional risk factors in these patients as well as the presence of anticardiolipin and anti-$\beta_2$glycoprotein I antibodies.[20] However antinuclear antibodies might also contribute to the accelerated atherosclerosis found in these patients. Further, the association of antinuclear antibodies with atherosclerosis raises the possibility that these antibodies play a part in atherogenesis or arteriosclerosis in other autoimmune and inflammatory states, such as vasculitides. As for other antibodies, the frequency of antinuclear antibodies is significantly higher in the elderly people (10-37%) than in the young (0-6%). [21] As coronary artery disease resulting from atherosclerosis is also found mostly in the elderly, antinuclear antibodies may be markers of advanced atherosclerosis or, alternatively, participate in its acceleration.

ATHEROSCLEROTIC PLAQUES

The collagenous portion of advanced atherosclerotic plaques, although the most prevalent, is also the most stable. In distinct contrast, the soft atheromatous

(lipid rich) component is particularly vulnerable to fissuring and rupture.[22-26] A collagen rich fibrous cap that varies in stiffness, strength, and thickness covers the lipid core. Typically the fibrous cap is thinnest at its corners. These areas are also the most heavily infiltrated with foam cells. Progressive extracellular lipid accumulation as well as the progressive growth of the lipid core towards the luminal aspect can destabilize atherosclerotic plaques.

THROMBOGENESIS

Occlusive thrombosis occurring at a site of the plaque rupture is multifactorial in nature. First, thromboresistance is already impaired and a procoagulant environment exists; second, there is a sudden change in the vascular geometry favouring platelet-vessel wall interactions[27]; and third, thrombogenic components within the plaque are directly exposed to circulating blood cells and coagulant proteins.

A classification of vascular injury preceding coronary arterial thrombosis has been proposed by Fuster and colleagues.[28] It is divided into three distinct types.

Type I:

Injury is localized to the vascular endothelium and develops in areas of eccentric intimal thickening, blood flow alterations, and vascular branch points. Platelets adhere to these areas, contributing to the plaque growth.

Type II:

Injury is more extensive, involving deep plaque structures. As a result, platelet adherence, activation, and thrombosis are provoked. With the appropriate procoagulant environment, type II injury can precipitate occlusive thrombosis.

Type III:

Injury is characterized by extensive plaque disruption with exposure of a variety of plaque constituents and subendothelial connective tissues. There is a strong propensity toward thrombosis. This type of injury is the most common among patients with acute myocardial infarction.[29]

The normal functioning hemostatic mechanism represents a dynamic, finely tuned balance between procoagulant and anticoagulant forces. Fibrin is a common component of coronary arterial atherosclerotic plaques, suggesting that coagulation is a participant in the atherosclerotic process. Even in the earliest stage of development, a uniform pattern of antifibrin antibody binding is observed, suggesting that fibrinogen derived from the infiltration of plasma is converted to fibrin within the intima of the vessels. Advanced atherosclerotic plaques contain a localized banded pattern of antifibrin antibody binding, consistent with direct incorporation of polymerized fibrin within the fibrous cap.[30 31]

Platelets also are important participants in atherosclerosis. They can support macrophage (foam cell) formation in cultured aortic smooth muscle cells.[32] It has also been observed that platelet depletion dramatically reduces the mitogenic response to vessel wall injury.[33]

Thrombin, a serine protease has many potential effects on atherosclerosis, can induce macrophage interleukin 1 synthesis, a cytokine capable of provoking smooth muscle cell proliferation and expression of leucocyte adhesion molecules on the endothelial surface.

A novel lipoprotein, LP (a), composed of LDL cholesterol and apolipoprotein (APO) a, has been shown by several investigative groups to compete with plasminogen for cell surface binding sites on monocytes, endothelial cells, and platelets.[34] [35] [36] LP (a) can also adhere to fibrin to augment the conversion plasminogen to plasmin. Lastly, LP (a) induces the expression and secretion of plasminogen activator inhibitor, reducing vascular fibrinolytic capacity and thromboresistance.

In acute myocardial infarction, occlusive intravascular thrombosis is the end result of profound local and systemic procoagulant factors. Some of the procoagulant states of importance are [37]

- Atherosclerotic coronary artery disease
- Hyperlipidemia
- Abnormal plasminogen activation

- Dysplasminogenemias
- Antiphospholipid antibody syndrome
- Antithrombin III deficiency
- Protein C deficiency
- Protein C resistance
- Protein S deficiency
- Malignancies
- Myeloproliferative disorders
- Homocystinuria
- Paroxysmal nocturnal hemoglobinuria
- Heparin (thrombocytopenia with thrombosis)
- Synthetic antifibrinolytic agents
- Prothrombin complex concentrates
- Cocaine
- Anabolic steroids
- Oral contraceptive agents

Autoimmunity has been implicated in a number of vascular processes including the initiation and the progression of atherosclerosis.[38] The major antigenic targets for autoantibodies during atherogenesis are the oxidized lipids

such as the oxidized low-density lipoprotein, heat shock proteins (HSP) 60/65, and phospholipids such as cardiolipin.[9, 38,39, 40]

Anticardiolipin antibodies have been associated with accelerated coronary atherosclerosis.[15, 41, 42] They are a heterogenous family of autoantibodies that are directed against the negatively charged phospholipids (such as cardiolipin, phosphatidylserine, phosphatidylinositol), phospholipid-protein complexes, or plasma proteins (such as β_2-glycoprotein I). [43]

Antiphospholipid antibodies are associated with an increased risk of arterial and venous thrombosis. Although myocardial infarction is not the most common type of arterial event that occurs with antiphospholipid antibodies, several prospective studies have provided evidence in favour of an increased risk with these antibodies.

The concept of a protein target for antiphospholipid antibodies evolved from a series of independent reports in 1990. It became clear that the binding of the antibodies to cardiolipin required a cofactor which was subsequently identified as β_2-glycoprotein I also known as apolipoprotein H.[44-46] β_2-glycoprotein I (β_2GPI) is a highly glycosylated single chain plasma protein composed of 326 amino acids with a molecular weight of 50 kDa that appears to be the major, but not the only cofactor for the recognition of anionic phospholipid by antiphospholipid antibodies. The protein is a member of the complement control protein or short consensus repeat superfamily. There is evidence that β_2-glycoprotein I itself may

be one of the major epitopes for antiphospholipid antibodies or may, in complex with phospholipids, form an antigenic site. The physiologic function of β_2GP1 has not yet been established, but it has been proposed that the protein may play a scavenging role for exposed anionic phospholipid after apoptosis.[47 48]

Following the discovery of the cofactor role for β_2GPI, additional candidate cofactors and antigenic targets were identified.[49 50]

ANTIGENIC TARGETS OF ANTIPHOSPHOLIPID ANTIBODIES

Major antigens:

- β_2-glycoprotein I
- Prothrombin

Others:

- Protein C
- Protein S
- Thrombomodulin
- Annexin V
- High/low molecular weight kininogen
- Factor XI

A number of studies demonstrated that the β_2 glycoprotein I-dependent binding to phospholipids could be used to discriminate between autoimmune aniphospholipid antibodies and those found in patients following infections.

Antiphospholipid antibodies present in autoimmune diseases are thrombogenic and β_2GPI dependent, as opposed to infection related antiphospholipid antibodies, which are thought less likely to be thrombogenic and are β_2GPI independent.[51] The presence of β_2GPI dependent antibodies was shown to be more specific for thrombosis than conventional anticardiolipin antibodies.

CLASSIFICATION OF ANTIPHOSPHOLIPID THROMBOSIS SYNDROMES

Antiphospholipid thrombosis syndrome associated with anticardiolipin antibodies is divided into one of six subgroups.[52] Although there appears to be no correlation with the type or the titer of anticardiolipin antibody and the type of the syndrome, the subclassification of thrombosis and anti cardiolipin antibody patients into these groups is important for therapy.

Type I syndrome

Deep venous thrombosis with or without pulmonary embolus

Type II syndrome

Coronary artery thrombosis

Peripheral artery thrombosis

Aortic thrombosis

Carotid artery thrombosis

Type III syndrome

Retinal artery thrombosis

Retinal vein thrombosis

Cerebrovascular thrombosis

Transient cerebral ischemic attack

Type IV syndrome

Mixture of types I, II, and III

Type IV patients are rare

Type V (fetal wastage) syndrome:

Placental vascular thrombosis

Maternal thrombocytopenia (uncommon)

Fetal wastage common in first trimester

Fetal wastage can occur in second and third trimester

Type VI syndrome:

Antiphospholipid antibody

No apparent clinical manifestation.

Most individuals with serum that reacts positively for antiphospholipid antibodies do not have systemic lupus, but may have a history of thrombosis. However, the antibodies may also appear transiently after tissue trauma, in infections, and as a response to exposure to certain drugs.

SITUATIONS IN WHICH ANTIPHOSPHOLIPID ANTIBODIES MAY BE DETECTED: [53]

Infections:

- Acute self-limiting infections
- Syphilis
- Malaria
- HIV infection
- Hepatitis C

Rheumatic and collagen vascular diseases

- Systemic lupus erythematosus
- Systemic sclerosis
- Rheumatoid arthritis
- Temporal arteritis
- Psoriatic arthropathy
- Sjogrens syndrome

Thrombotic disease

- Venous thromboembolic disease
- Peripheral arterial occlusion
- Microvascular thrombosis
- Myocardial infarction and ischaemic heart disease

- After coronary artery bypass graft surgery
- Valvular heart disease
- Renal vascular disease
- Pulmonary hypertension

Disorders of the nervous system and eye

- Thrombotic stroke
- Transient cerebral ischaemia and amaurosis fugax
- Sagittal-sinus thrombosis
- Ischaemic optic neuropathy
- Retinal venous occlusion
- Multi-infarct dementia
- Chorea
- Guillain-barre syndrome
- Transverse myelitis

Obstetric disorders

- Recurrent abortion
- Fetal growth retardation
- Early, severe pre-ecclampsia

With medication

- Phenothiazines

- Procainamide
- Hydralazine
- Phenytoin
- Quinidine

Miscellaneous

- Livedo reticularis
- Autoimmune thrombocytopenia
- Autoimmune hemolytic anaemia
- Bechet's syndrome
- Sickle-cell disease
- Intravenous drug abuse

ASSOCIATION OF ANTICARDIOLIPIN ANTIBODIES WITH VASCULAR INJURY

POSSIBLE MECHANISMS

- Endothelial activation
- Accelerated atherosclerosis
- Apoptosis
- Autoimmunity
- Genetic predisposition

ENDOTHELIAL ACTIVATION

Anticardiolipin have been documented in subendothelial cardiac deposits[54] and in intimal-medial borders in isolated human atherosclerotic plaques. The mechanism of anticardiolipin-associated vasculopathy includes interaction of endothelial cells with platelets and antiphospholipids, to promote a cascade of reactions yielding recurrent local thrombosis and intimal hyperplasia.

Anticardiolipin autoantibodies prompt a prothrombotic endothelial surface, [55] while the β_2GPI anticardiolipin antibody complex activates endothelium in vitro.[56]

Antiphospholipid antibody binding to endothelium induces in vitro up-regulation of adhesion molecules, such as intracellular adhesion molecule-1 and extracellular adhesion molecule-1, stimulated by an autocrine loop of interleukin-1β secretion.[57] Platelet-endothelium interaction mediated by anticardiolipin may alter thromboxane A2-prostacyclin balance, leading to enhanced thrombosis and vasoconstriction.[58]

Endothelin-1, which induces vasospasm and arterial occlusion, is released by the endothelium in response to antiphospholipid antibodies.[59]

A mechanism similar to heparin-induced thrombocytopenia has also been suggested for anticardiolipin associated vascular occlusion and thrombosis.[60] In addition to endothelin induced thrombosis, intimal hyperplasia plays a major part

in vascular occlusions. Endothelin, prompted by anticardiolipin, enhances endothelial cell proliferation in vitro.[61]

Initial endothelial damage exposes the anionic phospholipids that react with the phospholipid binding proteins, such as the β_2GPIor prothrombin. The simultaneous binding of anticardiolipin to cellular Fc receptor and phospholipid protein complex induces endothelial-platelet interaction resulting in thrombosis.

ACCELERATED ATHEROSCLEROSIS

An intriguing possible pathogenic role for anticardiolipin in vasculopathy is the cross reaction with oxidized low-density lipoprotein antibodies. Oxidized LDL is the principal lipoprotein found in atherosclerotic lesions, and it co-localizes with β_2GPI and immunoreactive lymphocytes.[62] Oxidized LDL binds to β_2GPI and that these complexes can be found in the blood stream of patients with various autoimmune and chronic inflammatory diseases such as the systemic lupus erythematosus, chronic renal disease, diabetes mellitus, as well as in patients with myocardial infarction.[63]

As phospholipids bear structural resemblance to LDL, anticardiolipin may cross-react with antioxidised LDL.[64] Each cardiolipin molecule contains four unsaturated fatty acids, highly susceptible to oxidation. Mice sera with high titers of oxidized LDL antibodies, bind cardiolipin effectively only after oxidation. Therefore oxidative events may also play a major part in anticardiolipin formation.[65] On the one hand, oxidized LDL aggravates in vitro the clinical

manifestations of antiphospholipids and on the other hand, atherogenic effect of human lupus sera in vitro may be mediated by LDL-containing immune complexes. LDL may also be involved in anticardiolipin associated vascular occlusion by inducing a prothrombotic state. LDL, itself may be a thrombogenic target of anticardiolipin, [66] and raised concentrations of lipoprotein (a) in patients with antiphospholipid antibodies may inhibit the fibrinolytic pathway.

APOPTOSIS

Hypercoagulability in persons with antiphospholipid antibodies may also be induced by apoptotic process. Alterations of the phospholipid phase of cell membranes during late apoptosis are immunogenic and associated with the production of antiphospholipid antibodies. These surface alterations also have an independent procoagulant activity.[67] Apoptotic cells may promote coagulation directly or via atherosclerotic plaque dislodgement.

Characteristic membrane blebbing, occurring in final stages of apoptosis may lead to the production of antiphospholipid antibodies and tissue factor procoagulant activity. Moreover, it has been claimed that via this pathway antiphospholipids may exert their hypercoagulability.[67] Apoptotic inflammatory cells, such as the macrophages and T cells, are found abundantly in atherosclerotic plaques and may induce plaque instability. Endothelial cell apoptosis may lead to loss of anticoagulant activity and increased leukocyte and platelet adhesion,

resulting in rapid progression of the atherosclerotic and calcification process.[67] On the other hand; antiphospholipid antibodies may enhance apoptosis, with nuclear DNA fragmentation, cell lysis, and membrane disruption.

AUTOIMMUNITY

Pathogenicity of antiphospholipids depends on the specificity, isotype, level, and duration of anticardiolipin.[68] Cross-regulatory roles of immunity and autoimmunity have recently emerged in vasculopathic and atherosclerotic processes.[69] A pre-requisite for this vascular autoimmunity is a humoral or cellular immune reactivity to self-antigens, [70] such as LDL turned immunogenic during oxidation and glycation and prompting T cell mediated immunity.

GENETIC PREDISPOSITION

Most anticardiolipin are species-specific recognizing only human plasma. They are associated with class 2 major histocompatibility complex and in particular the DQB1 locus and DRw53, and either DR4 (in Caucasians) or DR7 (in Latinos). Genetic factors probably play an important part in the thrombogenic mechanism of antiphospholipid antibodies.[54] Therefore, anticardiolipin may occur in genetically or immunologically susceptible patients after a common infection or after recurrent endothelial insults and local thrombosis.

DIAGNOSIS

The actual diagnosis of an ST segment elevation myocardial infarction does not rely on the electrocardiogram itself as the name might imply. The classic World Health Organization criteria[71] requires that two of the following three elements be present for the diagnosis of acute myocardial infarction:

1. A history suggestive of coronary ischemia for a prolonged period (>30 min)
2. Evolutionary changes on serial electrocardiograms suggestive of myocardial infarction
3. A rise and fall in serum cardiac markers consistent with myonecrosis

Only 2 out of 3 criteria are needed because of the wide variability in the pattern of patient presentation with acute myocardial infarction.

THE JOINT EUROPEAN SOCIETY OF CARDIOLOGY (ESC) / AMERICAN COLLEGE OF CARDIOLOGY (ACC) definition of myocardial infarction[72]

Criteria for acute, evolving or recent myocardial infarction

Either one of the following criteria satisfies the diagnosis for an acute, evolving or recent myocardial infarction.

1.Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following.

- Ischemic symptoms

- Development of pathologic Q waves on the electrocardiogram.
- ECG changes indicative of ischemia (ST segment elevation or depression)
- Coronary artery intervention (eg. coronary angioplasty)

2. Pathologic findings of an acute myocardial infarction.

MATERIALS AND METHODS

The present study was an observational study conducted on a total of 63 patients admitted into the intensive coronary care unit in the cardiology department at Govt. Stanley Medical College Hospital, Chennai. The period of study was from January 2005 to January 2006.

Selection criteria

Patients aged 55 years or younger, of either sex, who fulfilled the World Health Organization criteria[71] for the diagnosis of acute myocardial infarction, were included in the study. We decided to have a cut off age of 55 for patients enrolled in the study group, since the prevalence of positive ELISA antiphospholipid tests increases with age. Further more, the prevalence of anticardiolipin antibodies in apparently healthy elderly individuals aged more than 65 years ranged between 12-52%. [73,74] Equal number of age and sex matched controls were considered for comparison with our cases.

By a thorough history taking, the presence of conventional cardiovascular risk factors such as hypertension, smoking, dyslipidemia, diabetes mellitus, and family history of premature coronary artery disease were obtained. Blood samples were taken immediately after hospitalization to determine the presence of β_2-glycoprotein I dependent anticardiolipin antibodies. Laboratory recommendations regarding the transport of samples were strictly followed. The samples were analyzed at the Dept. of Immunology, Govt. Stanley Medical College, Chennai.

Since the serum lipid levels measured after the first 24 hours of acute myocardial infarction may be inconsistent and since, considerable numbers of patients were hospitalized beyond 24 hours, the lipid levels were not considered in the study.[75]

DETAILS OF LABORATORY ANALYSIS FOR THE PRESENCE OF β_2-GLYCOPROTEIN I DEPENDENT ANTICARDIOLIPIN ANTIBODIES

NAME OF THE TEST

It is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma.

PRINCIPLE

Highly purified cardiolipin is bound to microwells saturated with β_2-glycoprotein I. Antibodies to these antigens, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG and IgM immunologically bind to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end product. The intensity of this yellow color is measured photometrically at 450 nm.

The amount of color is directly proportional to the concentration of IgG and IgM antibodies present in the original sample.

STORAGE AND STABILITY

- Store the kit at 2-8 ^{0}C.
- Keep microplate wells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose the test reagents to heat, sun or strong light during storage and usage.
- Diluted sample buffer and wash buffer are stable for atleast 30 days when stored at 2-8 ^{0}C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of end point measurements at 450 nm
- Multi-channel dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionised water
- Graduated cylinder for 100 and 100 ml

- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8 ^{0}C for up to 5 days or stored at –20 ^{0}C for up to six months.
- Avoid repeated freezing and thawing of serum samples. This may result in variable loss of autoantibody activity. .
- Testing of heat-inactivated sera is not recommended.

PREPARATION OF THE REAGENTS

Preparation of the sample buffer

Dilute the contents of each vial of the sample buffer concentrate with distilled or deiodinised water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 ^{0}C for atleast 30 days after preparation or until the expiration date printed on the label.

Preparation of the wash solution

Dilute the contents of each vial of the buffered wash solution concentrate with distilled or deiodinised water to a final volume of 1000 ml prior to use. Store

refrigerated: stable at 2-8 ^{0}C for atleast 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples
2. Pipet 100 µl of calibrators, controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28 ^{0}C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA-SF: Standards A to F

P1,P2 **…**: Patient sample 1,2 ….

C1: Positive control

C2: Negative control

8. Dispense 100 µl of TMB (3,3', 5,5'-tatramethyl-benzidine) substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of stop solution to each well of the modules and incubate for 15 minutes at room temperature.

11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.
12. The developed color is stable for at least 30 minutes. Read optical densities during this time.

INTERPRETATION OF RESULTS

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-cardiolipin test

ANTI-CARDIOLIPIN ANTIBODIES

	IgG (GPL U/ml)	**IgM (MPL U/ml)**
NORMAL	<10	<7
POSITIVE	≥10	≥7

Quality control

This test is only valid if the optical density at 450 nm for positive control and negative control as well as for the calibrator A and F complies with the respective range indicated on the quality control certificate enclosed to each test kit. If any of these criteria is not met, results are invalid and the test should be repeated.

Calculation of the results

For anti-cardiolipin IgG and IgM, a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight-line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

STATISTICAL METHODS

Descriptive methods:

The distribution of the β_2-glycoprotein I dependent anticardiolipin antibodies between the cases and controls, as well as the distribution of the conventional cardiovascular risk factors in the study group were described using bar diagram and pie charts wherever appropriate.

Analytical methods:

The significance of the prevalence of β_2-glycoprotein I dependent anticardiolipin antibodies in the cases and the controls was analyzed using the statistical methods, Pearson Chi square test and Yates corrected Chi square test. The correlation between the conventional cardiovascular risk factors and β_2-glycoprotein I dependent anticardiolipin antibodies was done using the Chi square test. A ‘p’ value <0.05 was considered significant.

OBSERVATIONS AND RESULTS

In the present study, among the 63 cases, the age distribution ranged from 29-55 years, with a mean age of 44 years. Since age is a major risk factor for coronary artery disease (age>45 for males and >55 for females), the cases were subdivided into two groups with 45 as the line of demarcation between them. Acute myocardial infarction occurred predominantly in males when compared to females in our study.

TABLE 1

GENDER DISTRIBUTION OF THE STUDY POPULATION

	MALE **No (%)**	**FEMALE** **No (%)**	**TOTAL**
STUDY GROUP	57 (90.5 %)	6 (9.5 %)	63

TABLE 2

AGE DISTRIBUTION OF THE STUDY POPULATION

	AGE > 45 **No (%)**	**AGE ≤ 45** **No (%)**	**TOTAL**
STUDY GROUP	29 (46%)	34 (54%)	63

The presence of the conventional cardiovascular risk factors in the study population and its distribution was analyzed. Of the four risk factors (hypertension, diabetes mellitus, smoking, and family history of premature coronary artery disease) that were considered, smoking was widely prevalent in the study group.

TABLE 3

DISTRIBUTION OF DIABETES MELLITUS AMONG THE CASES

	DIABETICS **No (%)**	**NON DIABETICS** **No (%)**	**TOTAL**
STUDY GROUP	13 (20.6%)	50 (79.4%)	63

TABLE 4

DISTRIBUTION OF HYPERTENSION AMONG THE CASES

	HYPERTENSIVES **No (%)**	**NON HYPERTENSIVES** **No (%)**	**TOTAL**
STUDY GROUP	14 (22 %)	49 (77.8%)	63

TABLE 5

DISTRIBUTION OF SMOKING AMONG THE CASES

	SMOKERS **No (%)**	**NON SMOKERS** **No (%)**	**TOTAL**
STUDY GROUP	38 (60.3%)	25 (39.7%)	63

TABLE 6

FAMILY HISTORY OF PREMATURE CORONARY ARTERY DISEASE AMONG THE CASES

	FAMILY HISTORY OF PREMATURE CAD		TOTAL
	PRESENT	ABSENT	
STUDY GROUP	7 (11.1 %)	56 (88.9 %)	63

The antibody positivity was compared between the cases and the controls. The β_2-glycoprotein I dependant anticardiolipin antibodies were found to be positive in 17 patients in the myocardial infarction group whereas 3 people tested positive in the control group. The antibody positivity was statistically significant in the study group.

TABLE 7

DISTRIBUTION OF THE β2GPI DEPENDENT ANTICARDIOLIPIN ANTIBODIES AMONG THE CASES AND CONTROLS

	ANTIBODY POSITIVITY No (%)	**ANTIBODY NEGATIVITY No (%)**	**TOTAL**
STUDY GROUP	17 (27%)	46 (73%)	63
CONTROL GROUP	3 (4.8%)	60 (95.2%)	63

$\chi^2 = 11.7$ P = 0.001 statistically significant.

TABLE 8

ISOTYPE DISTRIBUTION OF ANTIBODY POSITIVITY IN CASES AND CONTROLS

ANTIBODY POSITIVITY	STUDY GROUP No	CONTROL GROUP No
IgG POSITIVITY	12	0
IgM POSITIVITY	4	3
IgG & IgM POSITIVITY	1	0

TABLE 9

IgG β2GPI ANTICARDIOLIPIN ANTIBODY IN CASES AND CONTROLS

	IgG POSITIVITY	IgG NEGATIVITY	TOTAL
CASES	13	50	63
CONTROLS	0	63	63
TOTAL	13	113	126

$\chi^2 = 14.5$ P = 0.001 statistically significant.

Isotype distribution of anticardiolipin antibodies namely IgG and IgM was studied in both the groups. IgG was the predominant isotype in the myocardial infarction group. A total of 12 patients were found to have IgG antibody positivity. Four patients showed IgM positivity while one had elevation of both the isotypes

The significance of the antibody isotypes was then analyzed across both the groups. It was found that the isotype IgG positivity was statistically significant in the study group when compared to the control group. The IgM positivity did not show any significant difference between the two groups.

TABLE 10

IgM β2GPI ANTICARDIOLIPIN ANTIBODY IN CASES AND CONTROLS

	IgM POSITIVITY	**IgM NEGATIVITY**	**TOTAL**
CASES	5	58	63
CONTROLS	3	60	63
TOTAL	8	118	126

χ^2_y = O.53 P = 0.47 Not statistically significant.

The conventional risk factors were then correlated with antibody positivity. Hypertension was the only factor that was significantly observed in the study group.

TABLE 11

HYPERTENSION AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY No	**ANTIBODY NEGATIVITY No**	**TOTAL**
HYPERTENSIVES	7	7	14
NON HYPERTENSIVES	10	39	49
TOTAL	17	46	63

$\chi^2 = 4.8$ P = 0.03 statistically significant

TABLE 12

DIABETES MELLITUS AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY No	**ANTIBODY NEGATIVITY No**	**TOTAL**
DIABETICS	4	9	13
NON DIABETICS	13	37	50
TOTAL	17	46	63

$\chi^2 = 0.12$ P = 0.73 Not statistically significant

TABLE 13

SMOKING AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY No	**ANTIBODY NEGATIVITY No**	**TOTAL**
SMOKERS	12	26	38
NON SMOKERS	5	20	25
TOTAL	17	46	63

$\chi^2 = 1.03$ P = 0.3 Not statistically significant

TABLE 14

FAMILY HISTORY OF PREMATURE OF PREMATURE CAD AND ANTICARDIOLIPIN POSITIVITY

FAMILY H/O PREMATURE CAD	**ANTIBODY POSITIVITY No**	**ANTIBODY NEGATIVITY No**	**TOTAL**
YES	2	5	7
NO	15	41	56
TOTAL	17	46	63

$\chi^2 = 0.01$ P = 0.9 Not statistically significant

TABLE 15

SEX AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY No	**ANTIBODY NEGATIVITY No**	**TOTAL**
MALE	15	42	57
FEMALE	2	4	6
TOTAL	17	46	63

$\chi^2 = 0.14$ P = 0.7 Not statistically significant

TABLE 16

AGE AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY No	**ANTIBODY NEGATIVITY No**	**TOTAL**
AGE > 45	10	19	29
AGE ≤ 45	7	27	34
TOTAL	17	46	63

$\chi^2 = 1.53$ P = 0.21 Not statistically significant

DISCUSSION

The correlation between raised antiphospholipid antibodies and arterial thromboembolism has been widely reported in the literature.[76-78] However, the link between the raised levels of antiphospholipid antibodies and increased incidence of myocardial infarction is less recognized.

Asherson[79] reported 13 young patients aged 20 to 52 years with myocardial infarction (4%) out of 300 antiphospholipid antibody positive patients. The possible pathogenic relationship between these antibodies and myocardial infarction remains controversial.

The rate of antiphospholipid antibodies in survivors of acute myocardial infarction ranges from 6% to 47%. [80-89] The prevalence of anticardiolipin antibodies in the healthy population ranges from 0% to 7.5%, depending on the particular assay and cut-off for positivity used, [90,91] and may reach 51.6% in the elderly.[73]

A number of studies demonstrated that the β_2 glycoprotein I-dependent (β_2GPI) binding to phospholipids could be used to discriminate between autoimmune antiphospholipids and those found in patients following infections. Antiphospholipid antibodies present in autoimmune diseases are thrombogenic and β_2GPI dependent, as opposed to infection related antiphospholipid antibodies, which are thought less likely to be thrombogenic and are β_2GPI independent.[51]

The presence of β_2GPI- dependent antibodies was shown to be more specific for thrombosis than conventional anticardiolipin antibodies.

In the population that we examined, the prevalence of β_2GPI-dependent anticardiolipin antibodies was 27%. This figure is significantly higher than the 4.8%, observed in the control group (P =0.001), which is within the anticipated normal range. We found significantly increased levels of IgG β_2GPI- dependent anticardiolipin antibodies (P =0.001) in our study group in comparison to the controls. In addition, a nonsignificant increase in the IgM β_2GPI-dependent anticardiolipin antibodies (P =0.47) was found in the infarction group when compared with the controls.

In our study, we found a notable, although not a significant difference in the frequencies of β_2GPI-dependent anticardiolipin antibodies between the males and females (26.3% versus 33.3%, respectively). However, the females constituted only about 9.5% of the study population.

When we divided our patients into two groups based on age (ie,group I-age>45, group II-age≤45) there was no significant difference in the frequencies of β_2GPI-dependent anticardiolipin antibodies between them.

We found significantly higher frequencies of β_2GPI dependent anticardiolipin antibodies among the patients with hypertension than in those without hypertension in the myocardial infarction group. However we did not find

any significant association between β_2GPI-dependent anticardiolipin antibody positivity and other conventional cardiovascular risk factors like diabetes mellitus, smoking, and family history of premature coronary artery disease.

Robin L.Brey et al [92] performed a nested case control study examining anticardiolipin as a risk factor for ischemic stroke and myocardial infarction by using stored frozen sera obtained from subjects enrolled in Honolulu Heart Program and were followed up for 20 years. The β_2GPI-dependent anticardiolipin of the class IgG was significantly associated with both incident ischemic stroke and myocardial infarction. Men with a positive assay tended to be older and had lower total cholesterol levels.

There are a good number of studies that looked into the prevalence of conventional anticardiolipin antibodies of isotypes IgG and IgM in the absence of β_2GPI. Eli Zuckerman et al [93] found a high prevalence of anticardiolipin antibodies in relatively young survivors (aged 65 or younger) of acute myocardial infarction. They also found out that a high titer of anticardiolipin antibodies served as the only independent risk factor for subsequent thromboembolic events or a reinfarction after an acute myocardial infarction. Logistic regression analysis of coronary risk factors in the antibody positive group failed to show a statistically significant association.

Jerzy Dropinski et al[94] studied the possible association between antiphospholipid antibodies and carotid intima-medial thickness in young

survivors of myocardial infarction. 24% had an elevated antiphospholipid levels. There was a correlation between high antiphospholipid levels and intima-media thickness (P=0.01). Among the coronary risk factors only hypertension and smoking correlated with intima-media thickening. Tsai RT et al[95] detected IgG and IgM anticardiolipin antibodies, each in 16.1% of myocardial infarction patients.

Ferlazzo B et al.[96] found significantly higher levels of anticardiolipin antibodies (P≤0.05) in 30% of patients with acute myocardial infarction and in 35% of angina pectoris. Chandrashekhara S et al [97] studied the incidence of anticardiolipin in various thrombotic settings. Among the 302 patients evaluated for thrombosis, 20.77% had elevated IgG anticardiolipin antibodies. Of the 58 patients who developed myocardial infarction, 13.79% tested positive for IgG anticardiolipin antibodies.

A prospective study undertaken by Seijas M et al [98] showed that the anticardiolipin antibodies were higher among the acute myocardial infarction patients than among the general population although the presence of such antibodies did not increase the risk of new post-infarction thrombotic events. Hamsten's study[80] of 62 young survivors of myocardial infarction resulted in 21% of patients with raised anticardiolipin titers on two or more occasions. The association between the raised anitcardiolipin antibodies and the new thrombotic events during the 36-64 months of follow up was of borderline significance.

STUDIES ON THE PREVALENCE OF CONVENTIONAL ANTICARDIOLIPIN ANTIBODIES

S.NO	STUDY	PREVALENCE OF ANTICARDIOLIPIN ANTIBODY POSITIVITY
1	Eli Zuckerman et al [93]	14% (either IgG or IgM)
2	Jerzy Dropinski et al [94]	24% (either IgG or IgM)
3	Tsai RT et al [95]	IgG: 16.1% IgM: 16.1% IgG or IgM: 1.8%
4	Ferlazzo B et al [96]	30% (either IgG or IgM)
5	Chandrashekhara S et al [97]	13.8% (IgG)
6	Seijas M et al [98]	12% (either IgG or IgM)
7	Singh K et al [99]	4.2% (either IgG or IgM)
8	Hamsten A et al [80]	21% (IgG)
9	Sletnes KE et al [81]	6.2% (IgG)
10	Phadke K V et al [100]	6.8% (IgG)

. The Italian registry of antiphospholipid antibodies evaluated the natural history and the risk for thrombosis in a cohort of 360 unselected patients followed for 4 years.[101] Asymptomatic patients with anticardiolipin antibodies had an incidence of thrombosis of 0.9% per patient-year.

There is evidence that β_2GPI itself may be one of the major epitopes for antiphospholipid antibodies. There are a few reports on the possible role of β_2GPI and anti-β_2 GPI antibodies in coronary artey disease. Farsi et al.[102] detected anti-β_2 GPI antibodies in a large proportion (29.7%) of coronary artery disease patients and in only 2.5% of controls.

Veres K et al[103] assessed the patients with acute coronary syndrome for the frequency and the type of antiphospholipid antibodies. The presence of antiphospholipid was also correlated with cardiovascular risk factors. Anti-β_2 GPI antibodies were found in 14.4% of acute coronary syndrome patients (21.2% in the myocardial infarction subgroup). However no difference was observed regarding the conventional anticardiolipin antibodies between the patients and controls. They also did not find a significant association of anti-β_2 GPI antibodies with any of the cardiovascular risk factors including hypertension, diabetes mellitus, smoking and lipid metabolism among their patients with acute coronary syndrome.

However the studies done by Sherer et al.[39] and Limaye et al.[42] did not support an association between anti-β_2 GPI antibodies and coronary artery disease. There are also reports that fail to disclose associations between antibodies against cardiolipin and myocardial infarction or ischemic heart disease.[81, 95, 99, 100]

Prospective controlled trials should shed light on this controversial subject. Two earlier prospective studies of dyslipidemic men reported that antibodies against cardiolipin and oxidized low-density lipoprotein are associated with an increased risk of myocardial infarction during a 5-year follow-up. Outi Vaarala et al[104] studied, whether the presence of anticardiolipin antibodies carries a risk of myocardial infarction in a prospective cohort of middle-aged dyslipidemic men participating in the Helsinki Heart Study, a 5-year coronary primary prevention trial with gemfibrozil.

The anticardiolipin antibody level (IgG) was significantly higher in patients than in controls ($p< 0.005$). Subjects with the antibody level in the highest quartile of distribution had a relative risk of myocardial infarction of 2 (95% confidence interval, 1.1 to 3.5) compared with the remainder of the population. This risk was independent of the confounding factors, such as age, smoking, systolic blood pressure, low-density lipoprotein, and high-density lipoprotein. Thus it was concluded that the presence of a high anticardiolipin antibody level was an independent risk factor for myocardial infarction or cardiac death.

The ability of anticardiolipin antibodies to predict myocardial infarction was investigated by Ruihua Wu et al[14] in a prospective nested case control study in which healthy 50-year-old men were followed up for 20 years. Raised levels of antibodies against cardiolipin at 50 years of age correlated positively with myocardial infarction 10 to 20 years later. IgG and IgA antibodies against cardiolipin were associated with myocardial infarction between 50 to 60 years of life. Moreover, higher antibody levels were noted in those who died from acute myocardial infarction in comparison to those who survived. Only IgG and IgA antibodies against cardiolipin were associated with future myocardial infarction, whereas IgM antibodies were not. The risk for myocardial infarction associated with these antibodies was independent of other well-established predictors such as supine blood pressure, serum LDL/HDL ratio, body mass index and smoking.

In conclusion, the association between anticardiolipin antibodies and myocardial infarction is relatively consistent except for some difference in opinion observed in few studies.[81,95,99,100] There may be several reasons for these discrepancies. First, there are only few prospective studies, whereas a majority of the studies looked into the survivors of myocardial infarction or patients with established ischemic heart disease. Second, there are differences in the study populations. Third, differences in the test techniques may be involved. The study we conducted supported an association between anticardiolipin antibodies and myocardial infarction.

CONCLUSION

The prevalence of $\beta_2$glycoprotein I-dependent anticardiolipin antibodies was found to be 27%.

IgG $\beta_2$glycoprotein I-dependent anticardiolipin antibodies was the most relevant isotype associated with acute myocardial infarction.

Hypertensives had a higher frequency of β2 glycoprotein I dependent anticardiolipin antibodies.

β2 glycoprotein I dependent anticardiolipin antibodies did not show a significant association with other conventional risk factors considered. (diabetes mellitus, smoking, and family history of premature coronary artery disease).

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PROFORMA

Name : Age/Sex :

IP NO : Address :

History & Presenting complaints

Chest pain :

Duration :

Breathlessness :

Palpitation :

DM :

HT :

Dyslipidemia :

Smoking :

Alcohol :

Family h/o premature CAD :

Drug history :

H/O recurrent thromboembolic events :

EXAMINATION

Pulse rate: BP:

General examination:

Cardiovascular system: Respiratory system:

Weight : Height : BMI :

INVESTIGATIONS

Total count : Differential count : P L E

Hb% : ESR :

CRP : VDRL :

Blood sugar : Urea :

Serum creatinine : Serum electrolytes :

Total CK :

CK – MB :

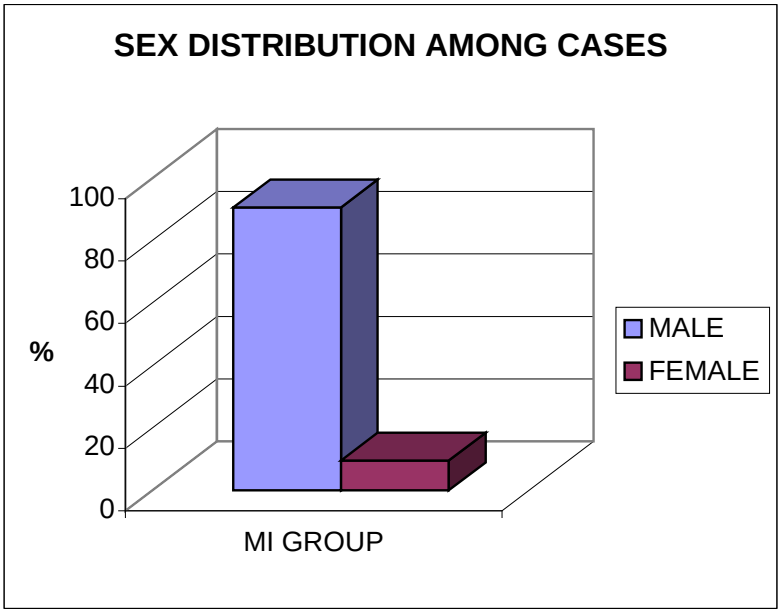
Electrocardiogram :

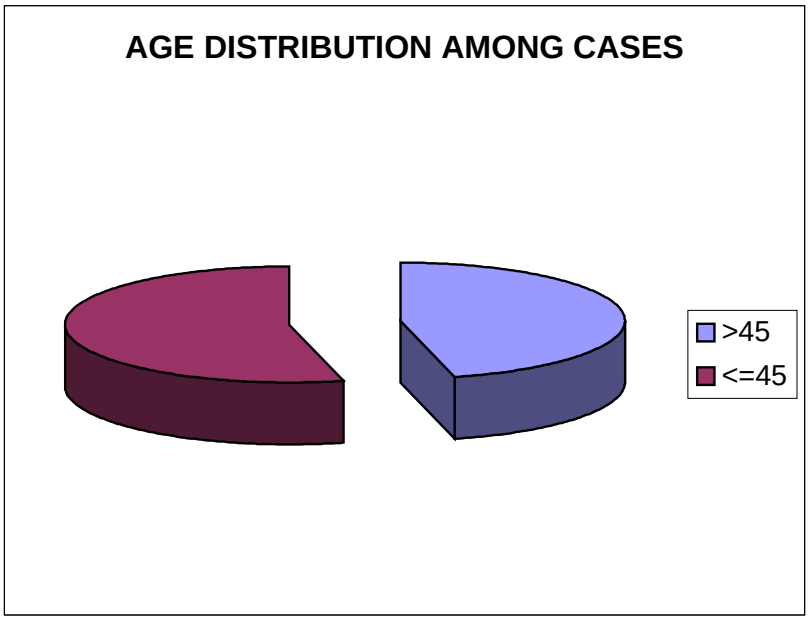
Echocardiogram :

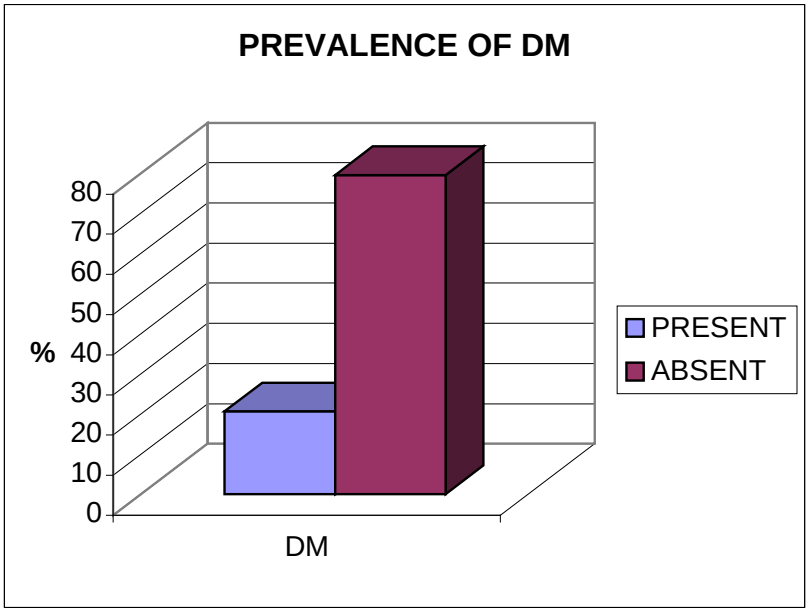
β_2 glycoprotein I-dependent anticardiolipin antibodies

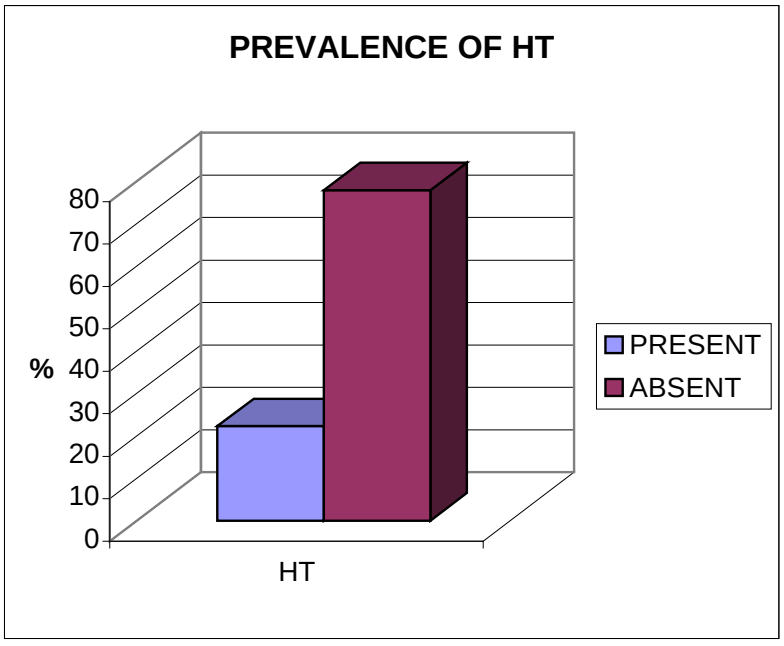
IgG :

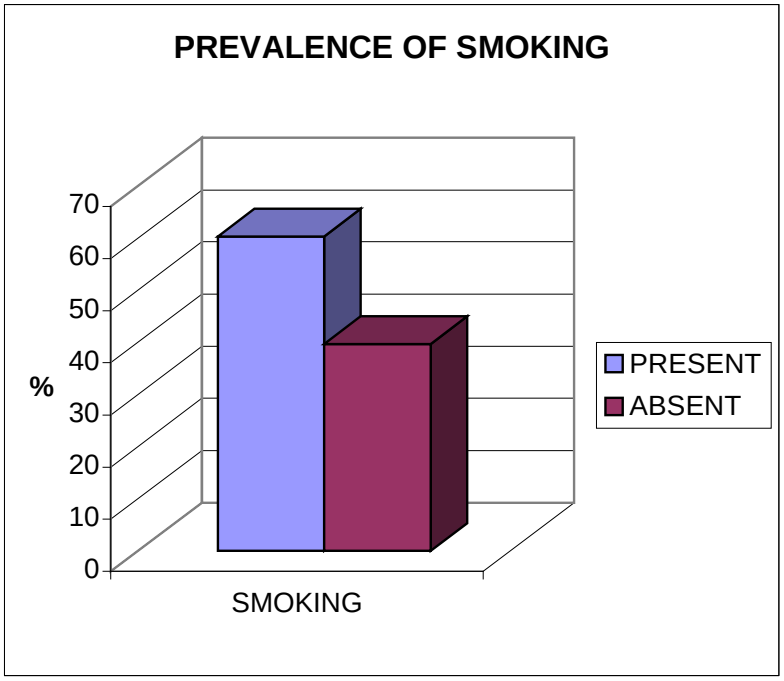
IgM :

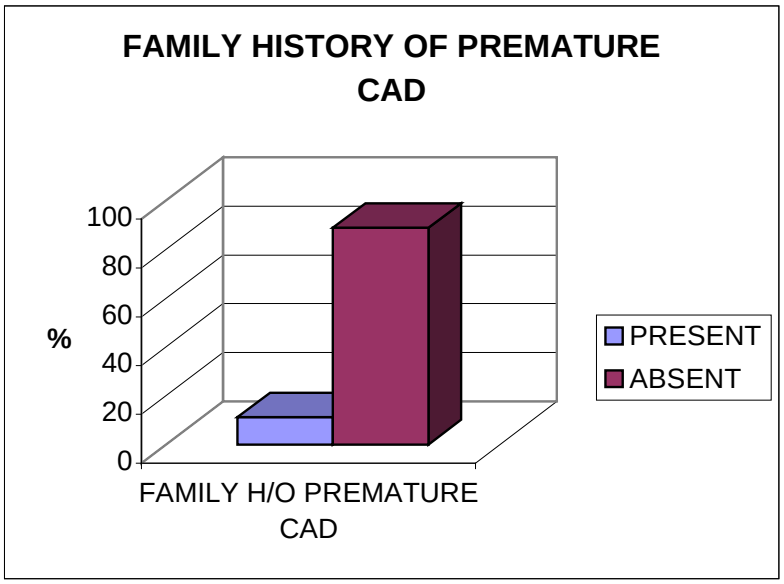

SEX DISTRIBUTION AMONG CASES
100
80
60
40
20
0
%
MI GROUP
MALE
FEMALE

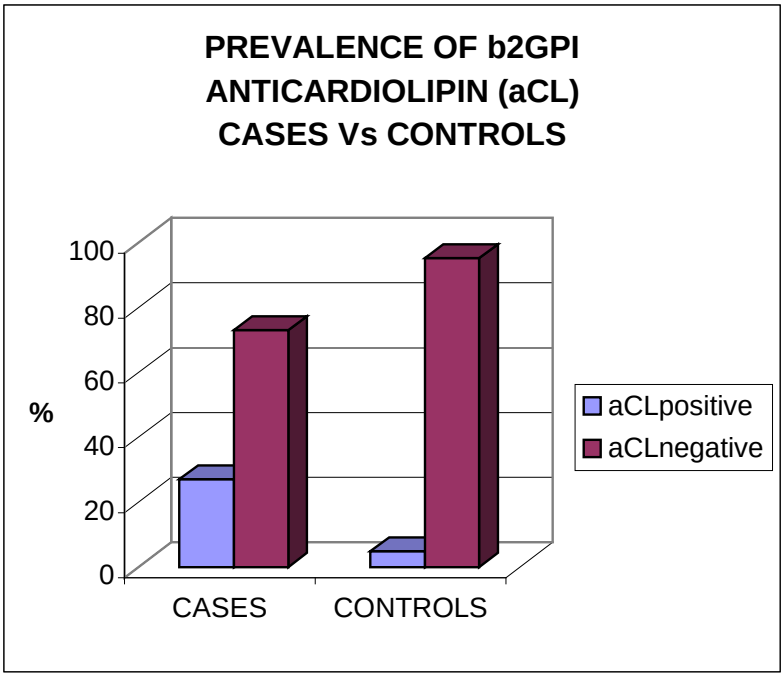

AGE DISTRIBUTION AMONG CASES
>45
<=45

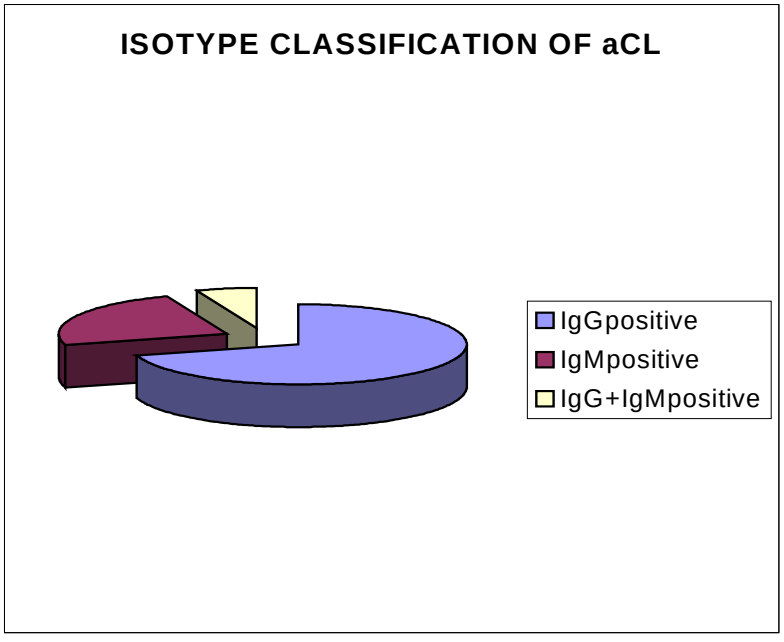

PREVALENCE OF DM
80
70
60
50
%
40
30
20
10
0
DM
PRESENT
ABSENT

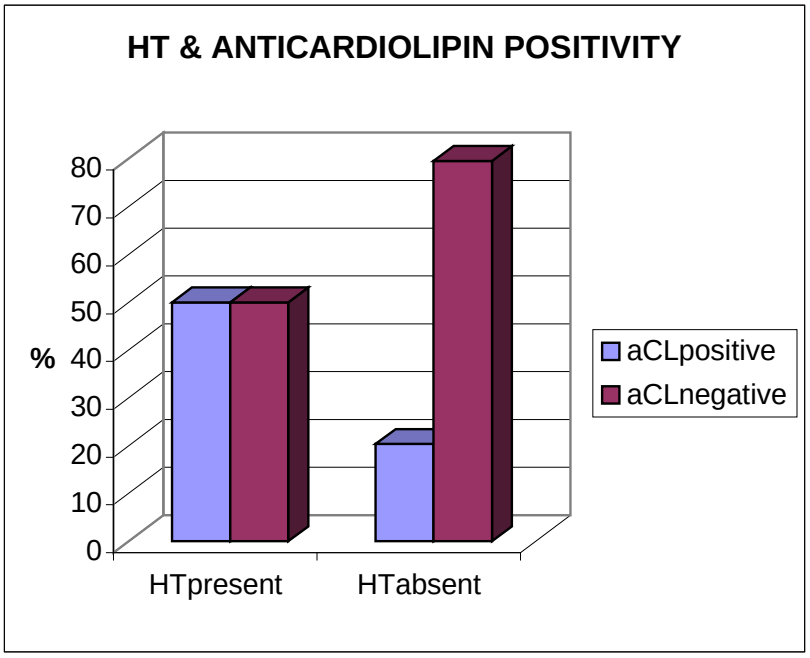

PREVALENCE OF HT
80
70
60
50
%
40
30
20
10
0
HT
PRESENT
ABSENT


PREVALENCE OF SMOKING
70
60
50
40
30
20
10
0
%
SMOKING
PRESENT
ABSENT


FAMILY HISTORY OF PREMATURE CAD
100
80
60
40
20
0
%
FAMILY H/O PREMATURE CAD
PRESENT
ABSENT


PREVALENCE OF b2GPI
ANTICARDIOLIPIN (aCL)
CASES Vs CONTROLS
100
80
60
40
20
0
%
CASES
CONTROLS
aCLpositive
aCLnegative


ISOTYPE CLASSIFICATION OF aCL
IgGpositive
IgMpositive
IgG+IgMpositive


HT & ANTICARDIOLIPIN POSITIVITY
80
70
60
50
%
40
30
20
10
0
HTpresent
HTabsent
aCLpositive
aCLnegative

S.NO	NAME	GROUP	AGE	SEX	IgG_ACL	IgM_ACL
1	DEVAN	2	49	1	0	0
2	PUNNIYAKODI	2	40	1	0	0
3	KUPPAN	2	55	1	0	0
4	GOWRISHANKAR	2	32	1	0	0
5	ASHOK KUMAR	2	52	1	0	0
6	SUBBAIYA	2	49	1	0	0
7	ABDUL RAHIM	2	45	1	0	0
8	SHANMUGAM	2	45	1	0	0
9	SYED MOHAMMED	2	52	1	0	0
10	VASANTH	2	37	1	0	0
11	SENTHIL	2	52	1	0	0
12	SHANKAR	2	38	1	0	0
13	NAGAJYOTHI	2	53	0	0	0
14	NAZEER	2	40	1	0	0
15	JOHN	2	50	1	0	0
16	RAJESH	2	51	1	0	0
17	NAGARAJ	2	50	1	0	1
18	KANNAPAN	2	48	1	0	0
19	BHUVANESHWAR	2	52	1	0	0
20	GANGADARAN	2	53	1	0	0
21	PRAKASH	2	44	1	0	0
22	MALATHI	2	48	0	0	0
23	SUNDAR	2	50	1	0	0
24	VASANTHA	2	50	0	0	0
25	MURUGAN	2	50	1	0	0
26	JEYARAJ	2	45	1	0	0
27	RAJKUMAR	2	52	1	0	0
28	JEYANTHI	2	38	0	0	0
29	MURALI	2	50	1	0	0
30	ABRAHAM	2	37	1	0	1
31	JEYARAJ	2	47	1	0	0
32	SURENDAR	2	54	1	0	0
33	KANNAN	2	31	1	0	0

34	SHANKAR	2	54	1	0	0
35	KUMAR	2	38	1	0	0
36	NATARAJAN	2	42	1	0	0
37	VENKATESH	2	33	1	0	0
38	SAKTHIVEL	2	45	1	0	0
39	JEGANNATHAN	2	38	1	0	0
40	SREENIVASAN	2	37	1	0	0
41	RANGAN	2	38	1	0	0
42	SIVALINGAM	2	42	1	0	0
43	JEYACHANDRAN	2	46	1	0	0
44	RAMAN	2	36	1	0	0
45	LINGAM	2	35	1	0	0
46	DEVENDRAN	2	50	1	0	0
47	SRIRAM	2	40	1	0	0
48	THAVASI	2	52	1	0	0
49	RAGU	2	52	1	0	0
50	GAYATHRI	2	35	0	0	0
51	KARUPPAN	2	53	1	0	0
52	SAMY	2	35	1	0	0
53	NARAYANAN	2	52	1	0	0
54	GANGA	2	45	0	0	0
55	ANNAMALAI	2	48	1	0	0
56	ARUL	2	38	1	0	0
57	RAMAN	2	38	1	0	0
58	VIJAYKUMAR	2	34	1	0	0
59	GOPAL	2	32	1	0	0
60	RAMALINGAM	2	42	1	0	0
61	MOORTHY	2	30	1	0	0
62	THANGAVEL	2	29	1	0	0
63	SUDAKAR	2	52	1	0	1

S.NO	NAME	GROUP	AGE	SEX	INFARCT	DM	HT	SMOKING	ALCOHOL	FAMILYHO	IgG_ACL	IgM_ACL	POSITIVE	AGE45
1	PUNNIYAKODI	1	49	1	ASMI	1	1	0	0	0	0	0	0.00	2.00
2	KUMAR	1	40	1	ASMI	0	0	1	1	0	0	0	0.00	1.00
3	GNANAMANI	1	55	1	AWMI	1	0	0	0	0	0	0	0.00	2.00
4	SRINIVASAN	1	32	1	AWMI	0	0	1	1	0	1	0	1.00	1.00
5	GNANANANDAN	1	52	1	AWMI	0	0	1	1	0	0	0	0.00	2.00
6	MOHAN	1	49	1	AWMI	1	1	0	0	1	1	0	1.00	2.00
7	NAZIMUDIN	1	45	1	IWMI	0	1	1	1	0	0	0	0.00	1.00
8	MANIVANNAN	1	45	1	IWMI	1	0	0	0	0	0	0	0.00	1.00
9	VENUGOPAL	1	52	1	IWMI	0	1	1	1	0	1	0	1.00	2.00
10	INDRA	1	37	0	ASMI	0	0	0	0	0	0	0	0.00	1.00
11	ISAC JAMES	1	52	1	IWMI/DMI	1	1	1	1	0	1	0	1.00	2.00
12	BALU	1	38	1	IWMI	0	0	0	1	0	0	0	0.00	1.00
13	BALARAMAN	1	53	1	IWMI/DMI	0	1	1	1	0	0	0	0.00	2.00
14	MURUGANANDAM	1	40	1	AWMI	0	0	1	1	0	1	0	1.00	1.00
15	BALAKRISHNAN	1	50	1	IWMI/DMI/RVMI	0	0	1	1	0	0	0	0.00	2.00
16	MOORTHY	1	51	1	AWMI	1	0	1	1	0	0	0	0.00	2.00
17	SUNDAR RAO	1	50	1	ASMI	1	1	0	1	0	0	0	0.00	2.00
18	BHERUDOSS	1	48	1	AWMI	1	1	1	0	0	0	1	1.00	2.00
19	SHABIR AHAMED	1	52	1	ILMI	0	1	0	0	0	1	1	1.00	2.00
20	KAJA MOIDEEN	1	53	1	IWMI/RVMI	0	1	1	1	0	1	0	1.00	2.00
21	GOVINDAN	1	44	1	ASMI	0	0	1	1	0	0	0	0.00	1.00
22	KANNIYAMMAL	1	48	0	IWMI	0	1	0	0	0	0	1	1.00	2.00
23	KRISHNAMOORTHY	1	50	1	AWMI	0	0	1	1	0	0	0	0.00	2.00
24	PECHIAMMAL	1	50	0	AWMI	0	0	0	0	1	0	1	1.00	2.00
25	RUSIYA	1	50	1	AWMI	0	0	1	0	0	0	0	0.00	2.00
26	MUNEER AHAMED	1	45	1	IWMI/DMI	0	0	0	0	1	0	0	0.00	1.00
27	VAIRAMANI	1	52	1	IWMI	0	0	1	1	0	1	0	1.00	2.00
28	BANUBEE	1	38	0	IWMI	1	0	0	0	0	0	0	0.00	1.00
29	PALANI	1	50	1	AWMI	0	0	1	1	0	0	0	0.00	2.00
30	ISMAIL	1	37	1	AWMI	0	0	1	0	0	1	0	1.00	1.00
31	ABDUL RAZAK	1	47	1	ASMI	0	1	1	0	0	0	0	0.00	2.00

32	SARAVANAN	1	54	1	IWMI/DMI	0	1	1	0	0	0	0	0.00	2.00
33	JANAKIRAMAN	1	31	1	ASMI	0	0	1	1	0	0	0	0.00	1.00
34	RAJENDRAN	1	54	1	ASMI	1	0	1	1	0	0	1	1.00	2.00
35	ANTONY	1	38	1	IWMI	0	0	0	0	0	0	0	0.00	1.00
36	RAMAMOORHTY	1	42	1	ASMI	0	0	1	1	1	0	0	0.00	1.00
37	SENTHIL KUMAR	1	33	1	AWMI	0	0	1	1	0	1	0	1.00	1.00
38	VELAYUDAM	1	45	1	ILMI	0	0	1	0	0	0	0	0.00	1.00
39	SEKAR	1	38	1	ASMI	0	0	1	1	0	0	0	0.00	1.00
40	MOORHTY	1	37	1	IWMI	0	0	0	1	0	0	0	0.00	1.00
41	DEVADOSS	1	38	1	AWMI	0	0	1	1	0	0	0	0.00	1.00
42	SHANKARDOSS	1	42	1	ILMI	0	0	0	0	0	0	0	0.00	1.00
43	MANOHARAN	1	46	1	IWMI/DMI	1	0	0	0	0	0	0	0.00	2.00
44	SELVAKUMAR	1	36	1	IWMI	0	0	1	1	0	0	0	0.00	1.00
45	MARIMUTHU	1	35	1	IWMI/DMI	0	0	0	0	1	0	0	0.00	1.00
46	SELVAM	1	50	1	ASMI	0	0	1	0	0	0	0	0.00	2.00
47	RAJENDRAN	1	40	1	AWMI	0	0	1	1	0	1	0	1.00	1.00
48	RAMASUBRAMANIY/	1	52	1	IWMI/DMI/RVMI	0	0	1	1	0	0	0	0.00	2.00
49	SAMSATH	1	35	0	AWMI	0	0	0	0	0	0	0	0.00	1.00
50	VENUGOPAL	1	53	1	ASMI	1	0	0	0	0	0	0	0.00	2.00
51	SHAHUL HAMEED	1	35	1	ILMI	0	0	1	1	1	0	0	0.00	1.00
52	JEYAPRAKASH	1	52	1	AWMI	1	1	0	0	0	0	0	0.00	2.00
53	THILAGAVATHY	1	45	0	IWMI/DMI/RVMI	0	0	0	0	0	0	0	0.00	1.00
54	BALARAMAN	1	48	1	ILMI/DMI	0	0	1	0	0	0	0	0.00	2.00
55	JEYARAMAN	1	38	1	AWMI	0	0	1	1	0	0	0	0.00	1.00
56	SEKAR	1	34	1	AWMI	0	0	1	1	0	0	0	0.00	1.00
57	BALAJI	1	32	1	IWMI/DMI	0	0	0	0	1	0	0	0.00	1.00
58	SEKAR	1	42	1	ASMI	0	0	0	1	0	1	0	1.00	1.00
59	JEGAN	1	30	1	IWMI	0	0	1	1	0	1	0	1.00	1.00
60	SARAVANAKUMAR	1	29	1	AWMI	0	0	1	1	0	0	0	0.00	1.00
61	AYYAMPERUMAL	1	52	1	AWMI	0	0	0	0	0	0	0	0.00	2.00
62	ELANGOVAN	1	38	1	ASMI	0	0	0	0	0	0	0	0.00	1.00
63	AMEER	1	40	1	IWMI/DMI	0	0	1	1	0	0	0	0.00	1.00

GROUP: 1- CASES, 2- CONTROLS
SEX: 0- FEMALE, 1- MALE
DM, HT, SMOKING, ALCOHOL,FAMILY HO: 0- ABSENT, 1- PRESENT
FAMILY HO: FAMILY HISTORY OF PREMATURE CAD
IgGaCL, IgMaCL: 0- NEGATIVE, 1- POSITIVE (aCL-anticardiolipin)
AGE<=45- 1, AGE>45- 2